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ESTIMATION OF THE CARNOSINE CONTENT OF DIFFERENT FIBRE TYPES IN THE MIDDLE GLUTEAL MUSCLE OF THE THOROUGHBRED HORSE

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SUMMARY

1. Skeletal muscle samples were obtained by needle biopsy from one of two depths of the m. gluteus medius in a group of twenty race-trained thoroughbred horses.
2. The content of carnosine was determined in each muscle sample, part of which was used for histochemical analysis. Fibres were classified as type I, type IIA or type IIB on the basis of the pH dependent lability of the myosin ATPase reaction.
3. Muscle samples with a higher type II fibre section area (FSA) have a higher carnosine content than those with a higher type I FSA.
4. Multiple linear regression analysis was used to estimate the mean carnosine content of individual fibre types. The results estimated a mean carnosine content in type I fibres of 54 mmol (kg dry muscle (DM))$^{-1}$, in type IIA fibres 85 mmol (kg DM)$^{-1}$ and in type IIB fibres 180 mmol (kg DM)$^{-1}$.
5. Based on the estimated values of single fibre carnosine content, there was close concordance between the estimated and the measured carnosine content of mixed fibre samples.
6. It would appear from this and other studies that carnosine has an important role as a physico-chemical buffer in equine middle gluteal muscle and that this is greatest in type IIB fibres, where it may account for up to 50% of physico-chemical buffering of H^+ produced by muscle in the pH range 7·1–6·5.

INTRODUCTION

Histidine-containing dipeptides such as carnosine, ophidine and anserine are important intracellular buffers and make a significant contribution to the buffering of hydrogen ions released within skeletal muscle during intense exercise. The buffering potential of histidine, which has a pK_a (−log of dissociation constant) of 5·97 (at 37 °C), is due to the acid–base behaviour of the imidazole ring. In the case

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of the dipeptides carnosine, ophidine and anserine, the relevant pK_a of the imidazole ring is increased to between 6·8 and 7·1 (at 37 °C), increasing the effectiveness of these compounds as buffers over the normal physiological pH range.

Highest contents of the histidine dipeptides are found in the muscles of animals exposed to prolonged periods of hypoxia, e.g. whales and sea snakes, and those adapted for high speed running (Davey, 1960). In a comparison of horse, dog and man, Harris, Marlin, Dunnett, Snow & Hultman (1990) concluded that the higher physico-chemical buffering in horse and dog was mainly attributable to the higher muscle dipeptide content.

Recently we have shown in the middle gluteal muscle of the horse that the highest carnosine contents are found in regions with the highest type II fibre section area, and that variation in the carnosine content accounts for most of the variation in physico-chemical buffering (Sewell, Harris & Dunnett, 1991). This initial study was undertaken in horses aged 6–11 years which is outside the normal range for horses involved in flat racing. The aim of the present study was therefore to examine the carnosine content of the middle gluteal muscle in relation to fibre composition in race-trained thoroughbred horses. From these analyses an estimate of the mean carnosine content of the different fibre types was derived.

This study has previously been presented in preliminary form (Sewell, Harris, Marlin & Dunnett, 1991).

METHODS

A single muscle biopsy was obtained from twenty, 2-year-old thoroughbred horses (18 colts, 2 fillies), by the needle biopsy technique of Bergstrom (1962). Horses were sampled towards the end of a race season during which nine of them had raced. All had been in training for five or more months. Topical and local anaesthetic was applied to the sampling area in preparation for the biopsy. None of the horses appeared to suffer any discomfort either during or after the taking of the biopsy samples. Samples were taken from the left middle gluteal muscle at a point one-third of the distance along a line running from the tuber coxae to the head of the tail and at a depth of either 4 cm (superficial) or 9 cm (deep). Each sample was divided into two portions.

Muscle histochemistry

A portion of the biopsy sample was mounted onto filter paper and frozen and stored in liquid nitrogen. These were used for the preparation of sections which were stained for myosin ATPase following pre-incubation at pH 4·5 to allow the demonstration of three fibre types (Brooke & Kaiser, 1970). To determine fibre proportion, an average of 345 (± 47) fibres were counted. To determine fibre area, eighty or more fibres of each type were measured wherever possible. In samples where there were less than eighty type I fibres in the section, as many fibres as were present were measured. Fibre areas were determined using a computerized image analysis system (Magiscan 1, Joyce-Loebl, Gateshead, UK). The percentage area occupied by each fibre type (fibre section area, FSA) was calculated according to Larsson & Ansved (1985).

Muscle biochemistry

The second portion of each biopsy sample was frozen and stored in liquid nitrogen, freeze-dried, dissected free of connective tissue and powdered. Neutralized perchloric acid extracts were prepared according to Harris, Hultman & Nordesjo (1974). Twenty microlitres were derivatized with *o*-phthalaldehyde, and carnosine was measured by high performance liquid chromatography. Carnosine was eluted with a methanol gradient and identified using fluorescence detection (Marlin, Harris, Gash & Snow, 1989). Quantitation was based on the comparison of the integrated peak areas with those from a range of known standards.

Values of carnosine content and fibre composition are presented as means $\pm$ S.D.

RESULTS

The results of the histochemical analysis of the muscle biopsy samples are presented in Table 1. Considerable differences existed between samples obtained at different depths. Mean muscle fibre composition of all samples combined was $16{\cdot}2 \pm 10{\cdot}8$ % type I and $83{\cdot}9 \pm 10{\cdot}8$ % type II. In two samples (both superficial) no type I fibres were detected. Type II muscle FSA ranged from 66 to 100 %, reflecting the high glycolytic capacity of this major propulsive muscle in the thoroughbred breed.

TABLE 1. Muscle fibre composition (% fibre section area) and carnosine content (mmol (kg DM)$^{-1}$) in superficial ($n = 10$) and deep ($n = 10$) samples of the middle gluteal muscle

	Superficial (4 cm)			Deep (9 cm)		
	Mean	S.D.	Range	Mean	S.D.	Range
Type I FSA	5·1	4·2	0–13	21·3	6·0	13–34
Type IIA FSA	28·9	6·1	19–42	46·0	5·5	35–53
Type IIB FSA	66·0	7·8	53–81	32·7	7·9	16–40
Carnosine	141	22	87–166	108	18	87–150

The carnosine content of biopsies ranged from 87 to 166 mmol (kg DM)$^{-1}$ (Table 1). Mean carnosine content was 125 ± 26 mmol (kg DM)$^{-1}$ for all samples ($n = 20$). This is higher than the mean carnosine content (109 ± 15 mmol (kg DM)$^{-1}$) reported by Marlin *et al.* (1989), but may be due to the fact that all of the horses sampled in the present study were young and highly trained.

Fibre composition and carnosine content

There was a positive correlation ($r = 0{\cdot}60$, $P < 0{\cdot}005$, $n = 20$) between carnosine content and type II muscle FSA. To estimate the carnosine content in the different fibre types multiple linear regression analysis was performed on nineteen values, omitting one point which was markedly removed from the mean trend.

The carnosine content of the different muscle fibre types was estimated according to the following mathematical model:

$$[\mathrm{Carn}]_{\mathrm{muscle}} = \left\{ [a\left(\frac{\mathrm{I\,FSA}}{100}\right)] + [b\left(\frac{\mathrm{IIA\,FSA}}{100}\right)] + [d\left(\frac{\mathrm{IIB\,FSA}}{100}\right)] \right\}, \quad (1)$$

where $[\mathrm{Carn}]_{\mathrm{muscle}}$ is the carnosine content (mmol kg^{-1} DM) of whole muscle, and a, b and d are constants estimating the carnosine content (mmol kg^{-1} DM) in type I, IIA and IIB fibres respectively.

Since

$$\frac{\mathrm{I\,FSA}}{100} + \frac{\mathrm{IIA\,FSA}}{100} + \frac{\mathrm{IIB\,FSA}}{100} = 1,$$

then eqn (1) can be rearranged as follows:

$$[\mathrm{Carn}]_{\mathrm{muscle}} = \left\{ [(a-d)\left(\frac{\mathrm{I\,FSA}}{100}\right)] + (b-d)\left(\frac{\mathrm{IIA\,FSA}}{100}\right)] + d \right\}. \quad (2)$$

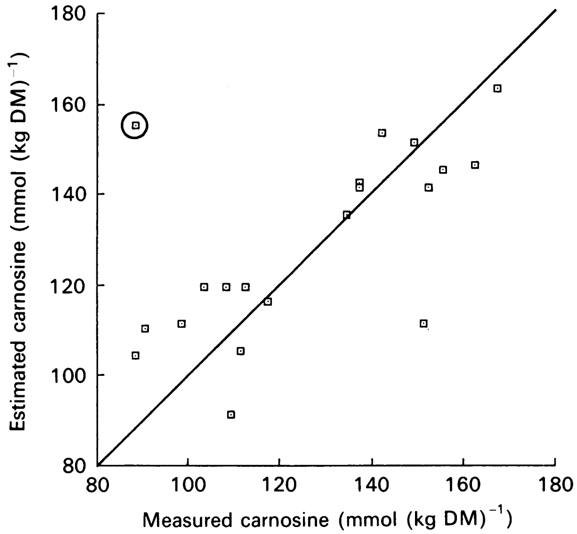

Fig. 1. A comparison of the carnosine content of muscle samples calculated from the observed FSA of each fibre type and the estimated content in the different fibre types, with the contents measured ($r = 0{\cdot}81$, $P < 0{\cdot}001$, $n = 19$). The drawn line represents the line of equality between estimated and observed values.

TABLE 2. Estimates of carnosine content (mmol (kg DM)$^{-1}$) in different fibre types

	Present study		Previous study	
Muscle fibre type	Mean	S.E.	Mean	S.E.
Type I	54	15	21	16
Type IIA	85	15	86	16
TYpe IIB	180	15	116	16

* Post-mortem muscle (Sewell *et al.* 1991). S.E. = standard error of the regression.

Equation (2) can also be expressed using type IIA and type IIB, and type I and type IIB as variables. Using eqn (2) the constants $(a-d)$, $(b-d)$ and d can be estimated from multiple linear regression analysis, from which a and b are calculated. The results showed a three- to fourfold variation in the carnosine content between the different muscle fibre types (Table 2).

From the estimates of carnosine content derived, the following mathematical model was constructed:

$$[\mathrm{Carn}]_{\mathrm{muscle}} = \left\{[54\left(\frac{\mathrm{I\,FSA}}{100}\right)+[85\left(\frac{\mathrm{IIA\,FSA}}{100}\right)]+[180\left(\frac{\mathrm{IIB\,FSA}}{100}\right)]\right\}. \quad (3)$$

This model was used to calculate the carnosine content $[\mathrm{Carn}]_{\mathrm{muscle}}$ of heterogeneous mixed muscle fibre samples. Estimated contents compared well with the actual measurements of carnosine content (Fig. 1, $r = 0{\cdot}81$, $P < 0{\cdot}001$, $n = 19$) indicating a concordance between the derived model and the *in vivo* condition. One point (circled in Figure 1) represents the results from the sample which was omitted from the fibre type estimations.

DISCUSSION

Systematic analyses of fibre composition in major propulsive muscles have been carried out in the horse (Bruce & Turek, 1985) and in man (Lexell, Henrikssen-Larsen & Sjostrom, 1983). Such studies have shown that different fibre types are not randomly distributed within the muscle, but tend to vary with depth. There is a greater proportion of type I fibres in the deeper regions of the muscle than in the superficial regions. This tendency is demonstrated for the present study, and was the reason for choosing the two depths.

In a previous study (Sewell *et al.* 1991), multiple linear regression analysis was used to estimate the carnosine content of the different fibre types of muscle samples obtained at post-mortem from different equine breeds. This resulted in estimates of carnosine content of 21 mmol (kg DM)$^{-1}$ as a mean for type I fibres, 86 mmol (kg DM)$^{-1}$ for type IIA and 116 mmol (kg DM)$^{-1}$ for type IIB fibres (Table 2). Compared with the present study, these earlier estimates are lower, and the ratio in contents between fibre types are different (especially between IIA and IIB fibres). This is probably due to the fact that in the present study, the horses sampled were younger, more highly trained and all of the thoroughbred breed.

Sprinting mammals perform high intensity exercise of short duration resulting in the production and accumulation of large amounts of lactic acid. The result is a rapid production and accumulation of protons, and a decrease in intracellular pH. The buffering of protons occurs through metabolic processes including phosphocreatine hydrolysis, loss of bicarbonate (Hultman & Sahlin, 1980) and through physico-chemical (or static) buffering by proteins, dipeptides and phosphates.

In equine muscle, physico-chemical buffering has previously been measured by titration and estimated to be approximately 118 mmol H^+ (kg DM)$^{-1}$ in the pH range 7·1–6·5, i.e. the millimoles of H^+ added per kilogram dry muscle that produces a pH change from 7·1 to 6·5 (Harris *et al.* 1990; Marlin & Harris, 1991). The contribution to this by non-dipeptide buffering ($\beta m_{\text{non-carn}}$) amounts to approximately 70 mmol H^+ (kg DM)$^{-1}$ (Sewell *et al.* 1991). This was estimated by measuring buffering capacity by titration (βm_{titr}) and deducting from this the specific buffering of carnosine (βm_{carn}) between the pH limits 7·1 and 6·5. $\beta m_{\text{non-carn}}$ appears to be a fairly constant figure between samples of varying fibre composition (Sewell *et al.* 1991) and between species (Harris *et al.* 1990).

The potential buffering capacity of different fibre types has been calculated in Table 3. These calculations assume a value of 70 mmol H^+ (kg DM)$^{-1}$ for non-dipeptide buffering. The relative contribution of carnosine to physico-chemical buffering is estimated to be only about 20% in type I fibres but as much as 46% in type IIB fibres. (A higher content of phosphocreatine in type II muscle fibres at rest compared with type I fibres suggested by data of Tesch, Thorstensson & Fujitsuka (1989) and Soderlund, Greenhaff & Hultman (1991) would only slightly increase non-carnosine physico-chemical buffering and thereby only slightly reduce the relative contribution of carnosine in type II fibres as estimated in Table 3.) The inference of a low carnosine content in type I muscle fibres is consistent with their recruitment during low intensity activity when accumulation of lactic acid is minimal and also with their much lower maximum velocity of shortening compared with type II fibres (Rome, Sosnicki & Goble, 1990).

Previous work by Bump, Lawrence, Moser, Miller-Graber & Kurcz (1990) has shown a higher carnosine content in the muscle of quarterhorses (39·2 μmol (g wet muscle)$^{-1}$) than in thoroughbreds (31·3 μmol (g wet muscle)$^{-1}$) and standardbreds (27·6 μmol (g wet muscle)$^{-1}$). The higher content of carnosine in quarterhorses is consistent with the greater percentage of fast twitch glycolytic fibres found in this breed (Snow & Guy, 1980).

TABLE 3. Calculated physico-chemical buffering capacities between the pH limits of 6·5 and 7·1

	Type I	Type IIA	Type IIB	Mixed muscle
$\beta m_{\text{non-carn}}$ (mmol H^+ (kg DM)$^{-1}$)*	70	70	70	70
βm_{carn} (mmol H^+ (kg DM)$^{-1}$)†	18	28	60	42
βm (mmol H^+ (kg DM)$^{-1}$)‡	88	98	130	112
βm_{carn} (as % of βm)	20	29	46	38
Carnosine content (mmol (kg DM)$^{-1}$)§	54	85	180	125‖

* Value assumed from Sewell *et al.* (1991).
† Specific buffering of carnosine calculated from the Henderson–Hasselbach equation.
‡ $\beta m_{\text{non-carn}} + \beta m_{\text{carn}}$.
§ Estimates from the present study.
‖ Measured value from the present study.

The higher content of histidine dipeptides in the muscles of animals exposed to prolonged periods of hypoxia, and those adapted for high speed running, suggests an adaptive response to the adverse effects of acidosis. The thoroughbred racehorse maintains speeds of around 17 m s^{-1} whilst racing over 1600 m. Remarkably, it would appear that such feats are easily surpassed by the pronghorn antelope which has been observed to run 11000 m at an average speed of 18 m s^{-1} (McNeill-Alexander, 1991). This is apparently achieved by an extraordinary capacity to consume and process enough oxygen without unique respiratory-system structures (Lindstedt, Hokanson, Wells, Swain, Hoppeler & Navarro, 1991). This capacity may be aided by an equally extraordinary ability of muscle to buffer hydrogen ion accumulation.

It would appear from this and other studies that carnosine has an important role as a physico-chemical buffer in equine middle gluteal muscle and that this is greatest in type IIB fibres. Such findings point to a functional adaptation in glycolytic fibres which may extend to other species.

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